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The Stomach Divalent Ion-sensing Receptor SCAR Is a Modulator of Gastric Acid Secretion*

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Divalent cation receptors have recently been identified in a wide variety of tissues and organs, yet their exact function remains controversial. We have previously identified a member of this receptor family in the stomach and have demonstrated that it is localized to the parietal cell, the acid secretory cell of the gastric gland. The activation of acid secretion has been classically defined as being regulated by two pathways: a neuronal pathway (mediated by acetylcholine) and an endocrine pathway (mediated by gastrin and histamine). Here, we identified a novel pathway modulating gastric acid secretion through the stomach calcium-sensing receptor (SCAR) located on the basolateral membrane of gastric parietal cells. Activation of SCAR in the intact rat gastric gland by divalent cations (Ca^{2+} or Mg^{2+}) or by the potent stimulator gadolinium (Gd^{3+}) led to an increase in the rate of acid secretion through the apical H^+,K^+-ATPase. Gd^{3+} was able to activate acid secretion through the omeprazole-sensitive H^+,K^+-ATPase even in the absence of the classical stimulator histamine. In contrast, inhibition of SCAR by reduction of extracellular cations abolished the stimulatory effect of histamine on gastric acid secretion, providing evidence for the regulation of the proton secretory transport protein by the receptor. These studies present the first example of a member of the divalent cation receptors modulating a plasma membrane transport protein and may lead to new insights into the regulation of gastric acid secretion.

Parietal cells secrete gastric acid in response to stimulation by either neuronally derived acetylcholine or via a biphasic endocrine pathway. In the endocrine pathway, release of gastrin from antral G cells leads to the activation of histamine-containing enterochromaffin-like (ECL)[1] cells (1). ECL cells then release histamine, which in turn initiates the direct insertion and activation of H^+,K^+-ATPase into the apical membrane of parietal cells. The exposure to histamine also causes a simultaneous rise in intracellular Ca^{2+}. Elevations in intracellular Ca^{2+} during this period have been associated with increased acid secretion and, as a result, have been used as an additional marker of the secretory process (2–4). Recently, a direct correlation between activation of the divalent cation receptor and Ca^{2+} entry into parietal cells has been established in which activation of the receptor by either divalent or trivalent ions leads to a rise in intracellular Ca^{2+} both from intra- and extracellular sites (5). Furthermore, the divalent receptor has been shown to modulate membrane Ca^{2+} channels and intracellular Ca^{2+} levels in G cells of the stomach (5). Recently, using calcium receptor-transfected human embryonic kidney cells, the first demonstration of calcium receptor modulation of a channel protein and an intracellular Ca^{2+} pathway was made (6). After activation of parietal cells by histamine, acid secretion occurs and the luminal pH of the gland decreases to approximately pH 1, leading to the efflux of protons from the gland lumen and resulting in a subsequent decrease in intragastric pH. However, during this massive flux of protons the intracellular pH of parietal cells remains stable at approximately pH 7 (3, 7). Any alteration in this regulatory acid secretory feedback loop leads to cell and tissue destruction and therefore has to be tightly regulated.

Two methods are commonly employed to counteract the overproduction of acid: (i) surgically, by elimination of the neuronal element (vagotomy) (8) or (ii) pharmacologically, either through histamine 2 receptor antagonists (9) or proton pump inhibitors (3, 10). Fine-tuning of the acid-secreting mechanism is still not completely understood and remains an important target for therapies to modulate gastric acid secretion.

The goal of the present study was to elucidate the physiological role of the stomach isoform of the calcium-sensing receptor (SCAR) on gastric acid secretion. We demonstrate that SCAR modulates acid secretion via regulation of the H^+,K^+-ATPase. Furthermore, this regulation of the transport protein appears to be independent of vesicular trafficking and the conventional hormonal pathways of acid secretion. Our studies present evidence for the first regulation of a membrane transport protein via a divalent cation receptor.

EXPERIMENTAL PROCEDURES

Sprague Dawley rats, 150–250 g (Charles River Laboratories, Wilmington, MA), were housed in climate- and humidity-controlled light-cycled rooms, fed standard rat chow, and allowed free access to water prior to investigation. Animals were killed with an overdose of pentobarbital, and the stomach was quickly removed. The fundus and antrum were isolated, sliced into 0.3-cm square sections, and washed several times in a standard, ice-cold, HEPES-buffered Ringer's solution (125 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1.2 mM $MgCl_2$, 32.2 mM HEPES,

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[1] The abbreviations used are: ECL, enterochromaffin-like; SCAR, stomach isoform of the calcium-sensing receptor; BCECF, 2′,7′-bis-(2-carboxyethyl)-5(6)-carboxyfluorescein; pH_i, intracellular pH.

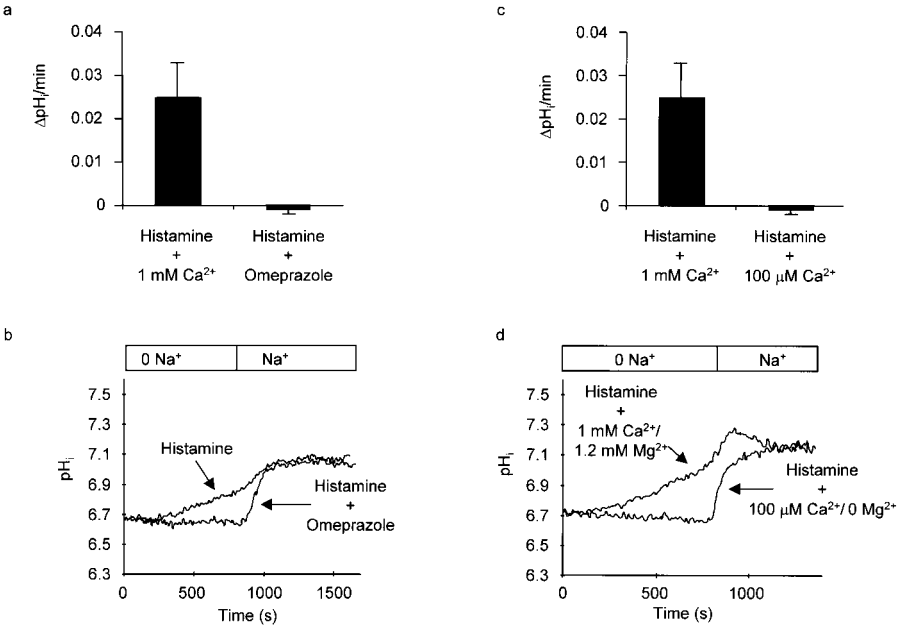

FIG. 1. **Regulation of SCAR-dependent acid secretion.** *a* and *b*, intracellular alkalinization stimulated by histamine in the absence of extracellular Na^+ is a function of H^+,K^+-ATPase because it can be blocked by the specific inhibitor omeprazole (100 μM) (n = 24 cells/4 glands). *c* and *d*, reduction of extracellular cations from 1 mM Ca^{2+} and 1.2 mM Mg^{2+} to 100 μM Ca^{2+} and 0 mM Mg^{2+}, respectively, abolished the stimulatory effect of histamine on intracellular alkalinization (H^+,K^+-ATPase activity) (n = 47 cells/5 glands).

and 5 mM glucose, pH 7.4 at 37 °C) to remove residual food particles. The tissues were then transferred to the stage of a dissecting microscope. Individual glands were isolated using a hand dissection technique as described previously (11). After isolation, the glands were allowed to adhere to coverslips precoated with Cell-Tak (Collaborative Research, Bedford, MA) and transferred to a thermostatically controlled chamber maintained at 37 °C on either a laser confocal microscope or on a video imaging system for the duration of the experiment.

Isolated gastric glands were incubated in a HEPES-buffered Ringer's solution containing 10 μM 2′,7′-bis(2-carboxyethyl)-5-(and 6)-carboxyfluorescein (BCECF)-acetomethyl ester (Molecular Probes, Eugene, OR) for 10 min. After loading, the chamber was flushed with Ringer's solution to remove de-esterfied dye. The perfusion chamber was mounted on the stage of an inverted microscope (Olympus IMT2) used in the epifluorescence mode with a ×40 objective. Following loading, the dye BCECF was successively excited at 440 nm and 490 nm and the resultant fluorescent signal was monitored at 535 nm using an intensified charge-coupled device camera. Data points were acquired every 8 s. The 490/440 intensity ratio data were converted to intracellular pH (pH_i) values using the high K^+/nigericin calibration technique (12).

Over the pH range of 6.5–7.5, fluorescence varied in a linear fashion with extracellular pH. Data are expressed as ΔpH/min. Acid extrusion was monitored in the absence of bicarbonate as intracellular alkalinization after the removal of Na^+ from the bath, which caused a reproducible and sustained intracellular acidification. Alkalinization rates (ΔpH_i/min) for the calculation of Na^+-independent pH_i recovery (H^+,K^+-ATPase activity) and Na^+-dependent pH_i recovery (Na^+/H^+ exchanger activity) rates were measured in the range of pH 6.80–6.85 and 6.85–7.0, respectively. All measurements for a series were measured at the same initial pH to maintain a constant intracellular buffering power for the calculation of recovery rates.

For intracellular Ca^{2+} measurements, 15 glands (n = 5 animals) were loaded with 10 μM of the Ca^{2+}-sensing dye Fluo-3 AM (Molecular Probes) in the chamber for 10 min at room temperature. Glands were then washed for 2 min with standard HEPES-buffered Ringer's solution to eliminate residual de-esterfied dye from the bath. Fluorescence was monitored with excitation at 488 nm and emission at 535 nm using a multiline argon laser coupled to the confocal microscope (Zeiss LSM 410). The fluorescence intensity was determined by measuring pixel values over each cell of interest before and after superfusion with 100 μM gastrin, 100 μM histamine, or 1.0 mM Gd^{3+}. Sequential frames were acquired at 2-s intervals, with each image comprising eight frames, on average. At least five parietal cells were analyzed in each experiment. Data were expressed in arbitrary fluorescence units (2).

All chemicals were obtained from Sigma; omeprazole was a kind gift from Astra Hässle AB, Mölndal, Sweden and was acid-activated prior to application to the glands. Activation of acid secretion via histamine stimulation was induced by preincubation of the glands for 15 min prior to the experiment. All data were summarized as mean ± S.E. and were analyzed by grouping measurements at baseline values and during experimental periods. Significance was determined using an unpaired Student's t test with $p < 0.05$ considered to be statistically significant.

RESULTS

High resolution video microscopy as well as laser confocal microscopy were employed to detect fluorochromic intensity changes within parietal cells in freshly isolated rat gastric glands. Measurements of acid secretion were conducted using the pH_i-sensitive dye BCECF to allow for a continuous online monitoring of pH_i during acid secretion. Changes in pH_i allowed us to observe activation or inhibition of the H^+,K^+-ATPase under resting as well as under stimulated conditions. We used several experimental approaches to test the decisive role of SCAR in gastric acid secretion. Challenging the cells with an acid load while inhibiting Na^+/H^+ exchange activity in the absence of bicarbonate allowed us to investigate only the apical H^+,K^+-ATPase as the sole H^+ extrusion pathway.

In the absence of histamine, no stimulation (Na^+-independent proton efflux) was observed (data not shown). Histamine (100 μM) exposure induced an alkalinization rate of 0.025 ± 0.001 pH unit/min. Fig. 1 shows that this recovery was completely inhibited by specific inhibitors of the gastric H^+,K^+-ATPase, either omeprazole (−0.001 ± 0.001 pH unit/min) (10) or SCH 28080 (Schering; −0.001 ± 0.001 pH unit/min) (13), demonstrating that the observed alkalinization was due to H^+ extrusion via the H^+,K^+-ATPase. Omeprazole or SCH 28080 had no effect on the pH_i of resting glands (data not shown).

Figs. 2 and 3*a* illustrate the effects of divalent ions on either the activation or the inhibition of H^+,K^+-ATPase activity via SCAR. Reduction of extracellular divalent cations (100 μM Ca^{2+}, 0 mM Mg^{2+}) effectively abolished histamine-induced alkalinization (−0.001 ± 0.001 pH unit/min). However, as shown in Fig. 3*a*, the trivalent cation Gd^{3+} stimulated H^+,K^+-ATPase activity both in the absence or presence of histamine and in the presence of low cations (100 μM Ca^{2+}, 0 mM Mg^{2+}) as shown by an increase in the rate of alkalinization (0.035 ± 0.004 pH unit/min). The stimulatory effect of Gd^{3+} was not mediated by the release of histamine from adjacent ECL cells, inasmuch as the H_2 receptor inhibitor cimetidine (100 μM) did not influence the effect of Gd^{3+} (0.038 ± 0.012 pH unit/min, Fig. 3*b*). However, inhibition of the H^+,K^+-ATPase by omeprazole abolished Gd^{3+}-induced alkalinization (−0.001 ± 0.002 pH unit/min), demonstrating that proton extrusion through the H^+,K^+-

ATPase was indeed responsible for the effect of Gd^{3+} (Fig. 3*a*). The onset of the Gd^{3+}-induced alkalinization occurred within 1–2 min after application, suggesting activation of H^+,K^+-ATPase pumps that were already inserted in the membrane. In addition, Fig. 2, *a* and *b* summarize the concentration dependence for both Ca^{2+} and Mg^{2+}, showing the activation and inhibition kinetics for SCAR on the basolateral membrane. By varying the level of total extracellular divalent ions, we could activate or inhibit the alkalinizing ability of the H^+,K^+-ATPase in parietal cells previously stimulated with histamine.

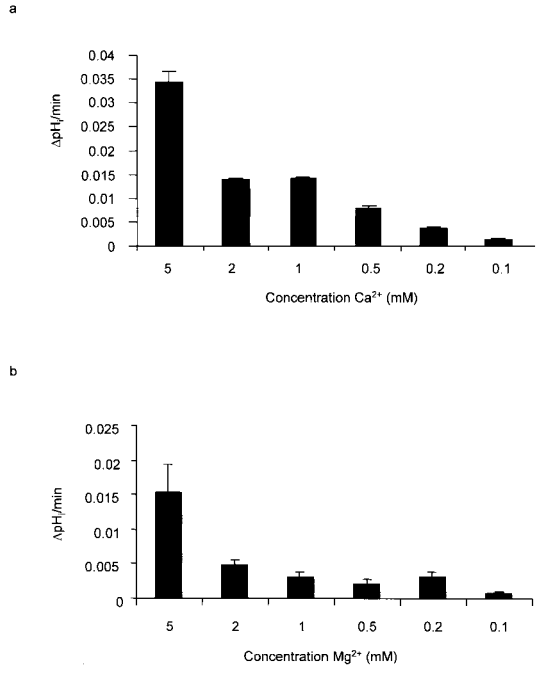

FIG. 2. **Calcium and magnesium dependence of SCAR.** *a*, calcium concentration dependence of H^+,K^+-ATPase activity (intracellular alkalinization) in the presence of histamine and the absence of Mg^{2+} (n = 20–30 cells/4–5 glands for each Ca^{2+} concentration). *b*, magnesium concentration dependence of H^+,K^+-ATPase activity in the presence of histamine and 100 μM Ca^{2+} (n = 20–30 cells/4–5 glands for each Mg^{2+} concentration).

Calcium levels increased upon stimulation with histamine under control conditions (Fig. 3*c*) as previously reported (2, 4, 14). Similar to our previous experiments with pH_i, the calcium response was inhibited by reducing total extracellular divalent ions (100 μM Ca^{2+}, 0 mM Mg^{2+}) even in the presence of histamine (Fig. 3*c*).

DISCUSSION

Our data demonstrate that SCAR is potentially important for the active secretion of gastric acid. Moreover, this receptor may modulate the conventional stimulatory hormonal pathways inasmuch as activation or inhibition of SCAR resulted in either an immediate activation or cessation of acid secretion (even in the presence of histamine), respectively. These studies suggest a possible mechanism for the tight regulation of pH_i of the parietal cell, a feature that has not been clearly defined in the classical model of acid secretion. The modulation of transporter activity via SCAR may provide a mechanism for maintaining pH_i during acid secretion. Despite the massive proton extrusion across the apical membrane that triggers a sharp decrease in luminal pH, pH_i remains almost constant during this stimulatory phase (7). This process requires simultaneous activation and inhibition of various transport moieties, such as the Na^+/H^+-exchanger (15, 16) and the Cl^-/HCO_3^--exchanger, as well as the recently described Na^+/HCO_3 co-transporter (17, 18). Modulation of Na^+/H^+ activity by SCAR was observed in this study but requires further investigation to elucidate its effects during histamine-regulated acid secretion in the presence of divalent ions. Accordingly, SCAR could potentially up- or down-regulate a variety of transporters on both the apical and basolateral membranes simultaneously to control acid secretion while preserving intracellular ion homeostasis. This tight control of both membranes would allow for the sustained flow of protons and Cl^- across the apical membrane while maintaining pH_i by either up- or down-regulating the remaining acid regulatory transporters on the basolateral and apical membrane. It should be pointed out that during the present studies we used a new method to assay directly for proton efflux, measuring pH_i after an acid load. This protocol allowed us to create an acid gradient from cell to lumen by removing Na^+ from the basolateral perfusate and to eliminate the regu-

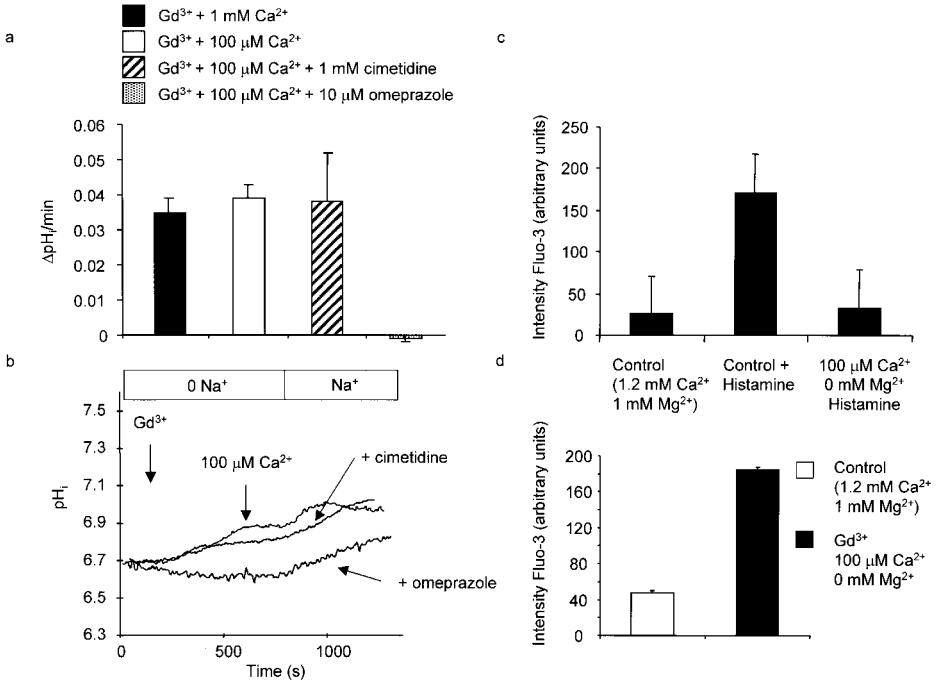

FIG. 3. **Mechanisms of SCAR-mediated alkalinization.** *a* and *b*, stimulation of H^+,K^+-ATPase activity with the divalent cation receptor agonist Gd^{3+} in the presence of both normal (1 mM Ca^{2+}, 1.2 mM Mg^{2+}) and reduced cations (100 μM Ca^{2+}, 0 mM Mg^{2+}). The effect of Gd^{3+} was not prevented by the histamine receptor type 2 antagonist cimetidine (1 mM) but was completely abolished by the H^+,K^+-ATPase inhibitor omeprazole (100 μM). This demonstrates that histamine release from adjacent ECL cells was not responsible for the activation of the H^+,K^+-ATPase and that the intracellular alkalinization was due to H^+,K^+-ATPase activity. *c*, effect of histamine in the presence of normal cations (1 mM Ca^{2+}, 1.2 mM Mg^{2+}) and reduced cations (100 μM Ca^{2+}, 0 mM Mg^{2+}) on intracellular Ca^{2+} levels. Reduction of cations abolished the histamine-induced increase in Ca^{2+} (n = 15–20 cells/4–5 glands). *d*, effect of Gd^{3+} on intracellular Ca^{2+} under reduced extracellular cations (100 μM Ca^{2+}, 0 mM Mg^{2+}, n = 15–20 cells/5–6 glands).

latory role of the Na^+/H^+ exchanger. We then monitored the efflux of protons (rate of alkalinization) via the Na^+-independent, omeprazole-sensitive H^+,K^+-ATPase. Although removal of Na^+ could result in a potential rise in intracellular Ca^{2+} (19) and activation of H^+,K^+-ATPase, we demonstrate in our studies instead that removal or addition of extracellular divalent ions appeared to be the trigger for acid secretion. Removal of Na^+ in non-histamine-stimulated glands in the presence or absence of divalent ions failed to activate acid secretion, whereas addition of Gd^{3+} caused an increase in acid secretion even at low levels of extracellular Ca^{2+}. As the effects on acid secretion elicited by SCAR appear to be linked to the levels of extracellular divalent or trivalent ions, we suggest that this important regulatory pathway may even overcome the histamine-related stimulation of acid secretion.

Moreover, SCAR could play an important role linking gastric acid secretion to the metabolic state. Modulation of Ca^{2+}-sensing receptors by amino acids has recently been reported and could provide the link between protein intake and gastric acid secretion (20). Similarly, hypercalcemia as a result of malignancy or hyperparathyroidism is accompanied by increased gastric acid secretion via a process that remains unidentified (21). SCAR could indeed regulate not only direct Ca^{2+} reabsorption but could at the same time lead to changes in the proton extrusion rates along the gastric glands, which has profound influences on intestinal Ca^{2+} absorption and whole body calcium content (22).

The identification of interactions of SCAR with proton efflux and cell ionic homeostasis suggest that divalent cation receptors may accomplish the regulation of total body Ca^{2+} homeostasis by interactions of the receptor with ion transporters or channels on the cell membranes. This feedback regulation could allow the divalent cation receptors to modulate Ca^{2+} reabsorption by varying the rate of proton efflux from the cell, which in turn would influence ionized Ca^{2+} levels.

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